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HEMATOLOGIC AND IMMUNOLOGIC STUDIES OF HUMANS EXPOSED TO SO_2

by

Mirdza L. Peterson, Shirley Harder, Delores Elliott,
Velva Milholland, Geraldine Orlando, Beth Margolis, and Dennis House

Human Studies Division
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, North Carolina 27711

HEALTH EFFECTS RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
RESEARCH TRIANGLE PARK, NORTH CAROLINA 27711

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No statistically significant changes were seen in s-IgA, blood erythrocytes or immunologic parameters examined. A possibly significant decrease was found in monocytes 48 hours following SO_2 exposure, but this recovered after 72 hours. A stimulatory effect (not statistically significant) was noted at 48 and 72 hours post-SO_2 in s-IgA and in lymphocyte numbers bearing receptors for the Fc portion of IgG, while a decrease occurred in active T-lymphocytes with receptors for sheep red blood cells.

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FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The Health Effects Research Laboratory, Research Triangle Park, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human subjects. These studies address problems in air pollution, non-ionizing radiation, environmental carcinogenesis, and the toxicology of pesticides as well as other chemical pollutants. The Laboratory develops and revises air quality criteria documents on pollutants for which national ambient air quality standards exist or are proposed, provides the data from registration of new pesticides or proposed suspension of those already in use, conducts research on hazardous and toxic materials, and is preparing the health basis for non-ionizing radiation standards. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the administrator to assure the adequacy of health care and surveillance of persons having suffered imminent and substantial endangering of their health.

Controlled exposure air pollution research with human subjects is difficult research, especially when information is needed concerning the effects of very low levels of pollutants. The special data quality control program in use at the Clinical Studies facilities of the Health Effects Research Laboratory provide us with an excellent opportunity to make such studies. In the case reported herein, there was a specific need for highly credible data on effects on normal human subjects of 0.75 ppm sulfur dioxide with measurement of as broad a spectrum as possible of potential health parameter decrements. This research was performed in response to that need and will serve to fill a gap in available data used to assess the hazards associated with exposure to the oxides of sulfure.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

ABSTRACT

Immunologic and hematologic parameters were used to evaluate the effects in humans of a single 2 hour exposure to either clean air or 0.75 ppm SO_2. Venous blood samples were obtained before, immediately after, and 24, 48, and 72 hours following the end of exposure. Parameters studied included complete blood counts, enumeration of lymphocyte populations using surface membrane receptor markers, evaluation of lymphocyte mitogen stimulated response, and concentration of secretory immunoglobulin A (s-IgA) content of nasal washings.

No statistically significant changes were seen in s-IgA, blood erythrocytes or immunologic parameters examined. A possibly significant decrease was found in monocytes 48 hours following SO_2 exposure, but this recovered after 72 hours. A stimulatory effect (not statistically significant) was noted at 48 and 72 hours post-SO_2 in s-IgA and in lymphocyte numbers bearing receptors for the Fc portion of IgG, while a decrease occurred in active T-lymphocytes with receptors for sheep red blood cells.

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The protocol was approved by the Committee for Protection of Human subjects of the University of North Carolina, School of Medicine, Chapel Hill, North Carolina.

SECTION 1

INTRODUCTION

Sulfur dioxide (SO_2) is one of the most commonly encountered atmospheric pollutants (Rall, 1974). Environmental exposure of humans to SO_2 has been associated with impairment of pulmonary function (Ferris et al., 1971, 1973, 1976); increased incidence of acute respiratory disease (Van der Lende et al., 1975; Lawther et al., 1970); higher prevelance of respiratory disease symptoms and exacerbation of chronic respiratory disease (Lunn et al., 1967, 1970; Douglas and Waller, 1966). It has been shown that 95%-98% of the inhaled SO_2 is absorbed by the nasal mucosa (Speizer and Frank, 1966; Frank et al., 1969), and after a brief initial period, most of the pollutant passes directly into the blood stream without reaching the lung (Anderson et al., 1974) where it dissociates into sulfite and bisulfite ions (Yokoyama et al., 1971). The sulfites react with disulfide bonds in plasma proteins to form thiosulfonates (Gunnison and Benton, 1971), which remain protein bound as long as eleven days after exposure (Gunnison and Palms, 1973). Bisulfite, which requires no metabolic activation, reacts with a large number of cellular components (Shapiro, 1977) including reactions with nucleic acids (Hayatsu and Miller, 1972). More direct evidence that SO_2, or products formed when SO_2 reacts with physiological material may have a detrimental effect upon cell-mediated immunity, has been provided by the studies of Schneider and Calkins (1971). These investigators found significant reductions in growth and DNA synthesis of human lymphocyte cultures exposed in vitro to SO_2.

Because of the research that indicates that SO_2/sulfite affects the DNA molecule, it was of interest to determine if this agent would affect the cellular elements of blood from humans exposed to SO_2. The study described here incorporates immunologic and hematologic parameters to measure the effect on peripheral blood cells of humans exposed for 2 hours to 0.75 ppm SO_2 or to clean air.

SECTION 2

MATERIALS AND METHODS

SUBJECT SELECTION

Male volunteers between the ages of 19 and 27 were studied after informed consent was obtained. Twenty-eight subjects completed a comprehensive medical questionnaire and were examined by the physician. None of the subjects smoked or had a history of cardiac disease, allergy, or infection. No subject had used any medication for at least 1 week prior to testing.

EXPOSURE PROTOCOL

Exposures to SO_2 or clean air took place in an airtight chamber described in detail by Strong (1978). During exposure, SO_2 was maintained at 0.75 ppm SO_2, ± 0.04 ppm, relative humidity at 60%, and temperature at 22.2°C. Subjects rested, except for a single 15 minute exercise period on a treadmill at 4 mph with a grade of 10% that began 45 minutes into the exposure. Treatment of subjects in the control cohort exactly duplicated that of the experimental cohort with the exception that exposure was to clean air rather than to SO_2.

Venous blood samples were taken prior to, and immediately following each exposure (for hematologic tests only) and again at 24, 48, and 72 hours post-exposure. All samples were processed immediately following collection.

HEMATOLOGIC ANALYSES

The hematologic analyses were made on blood containing EDTA as described by Henry (1979). A Coulter Counter (Model ZBI, Coulter Electronics, Hialeah, Florida) was used to determine red blood cell (RBC) and white blood cell (WBC) counts, hematocrit (HCT), and mean cell volume (MCV). Hemoglobin (Hgb) concentration was measured using a hemoglobinometer (Coulter Electronics). Cells were differentiated on Wrights' stained blood smears using a Honeywell 1000 analyzer (Honeywell, Inc., Denver, Colorado).

Lymphocyte Transformation

The mitogen-stimulated proliferative response of lymphocytes was performed as described by Oppenheim and Schecter (1976). Peripheral-blood mononuclear cells were isolated from heparinized blood (20 units/ml) by centrifugation over lymphocyte separation medium (LSM-Litton Bionetics, Kensington, Maryland). The cells were washed twice in RPMI-HEPES containing

5% heat-inactivated fetal calf serum (FCS), 2 mM L-glutamine, 50 µg of streptomycin and 50 units of penicillin per ml (Grand Island Biological Co., Grand Island, New York).

The cells were resuspended to 1 x 10^6 cells per ml in RPMI 1640-HEPES + 20% FCS. Cell suspensions typically contained 92 to 98% mononuclear cells with greater than 95% viability as determined by Trypan Blue exclusion. One hundred microliters of the leukocyte suspension was added to each well of a microtiter plate (Falcon Micro Test II, Oxnard, California). The control wells received medium only. The cells were stimulated with optimal concentrations (100 µl/well) of phytohemagglutinin (PHA; 2 µg/ml; Burroughs Welcome Co., Research Triangle Park, North Carolina) or pokeweed mitogen (PWM; 2.5 µl/ml; Grand Island Biological Co., Grand Island, New York). All cultures were prepared in triplicate and incubated at 37°C for 5 days in a humidified atmosphere with 5% CO_2. Twenty-two hours before harvesting, all cultures received 0.5 µCi of 2-^{14}C thymidine (specific activity, 51.6 mCi per mmol; New England Nuclear, Boston, Massachusetts). Cells were harvested and the uptake of ^{14}C thymidine by the cells was determined by means of scintillation counting. Data were expressed as counts per minute (cpm).

Rosette Formation

The details of this method have been described by Wybran et al. (1973). Briefly, mononuclear cell suspensions were prepared as described for the lymphocyte transformation studies. The cell layer was removed and washed twice in Hanks' balanced salt solution (HBSS; Grand Island Biological Co., Grand Island, New York). The monocytes were removed by adherence to plastic by suspending the cell suspension in RPMI 1640 medium containing 5% FCS and dispensing into a sterile, plastic Petri dish and incubating for 30 minutes at 37°C on a low speed rotary shaker. Cells were then washed, counted, and diluted to a concentration of 2 x 10^6 cells per ml.

For detecting active T-cell sheep erythrocyte rosette formation (TEa), lymphocytes were mixed with an equal volume of 0.5% suspension of sheep erythrocytes in HBSS and incubated for 1 hour at 37°C. This mixture was centrifuged for 5 minutes at 500 g, gently resuspended, and the number of rosettes formed by 200 lymphocytes was counted in a hemacytometer.

Fc receptor bearing lymphocytes were enumerated by incubating the lymphocyte suspension overnight at 4°C with equal amounts of human erythrocytes (group O) sensitized with IgG antibody. A lymphocyte with three or more erythrocytes attached was counted as a rosette. The percentage of rosettes was converted to absolute numbers and expressed as rosette forming cells (RFC).

COLLECTION, PREPARATION AND ANALYSIS OF NASAL WASHINGS FOR IgA

With the subject sitting upright and the neck hyperextended, 5 ml of pre-warmed (37°C) physiological saline (0.9%) was instilled into each side of the nasal cavity. The subject then leaned forward and forcibly expelled the washings into a sterile, disposable plastic cup. Washings from both nasal cavities were pooled and refrigerated overnight at 4°C to permit all globulin to be eluted from the threads of mucus. Twenty-four hours later, the specimen was centrifuged at 1200 rpm for 5 minutes, the mucus was removed, and the remainder was concentrated using an Amicon Microconcentrator (Amicon Corp., Lexington, Massachusettes) and then frozen at -20°C. The quantitation of IgA was performed using the FIAX™ method of immunofluorescence as described by Baier (1975). The values were expressed as mg/dl for statistical analysis.

STATISTICAL ANALYSIS

For each of the 17 hematologic and immunologic parameters, multivariate analysis of variance was used to test the hypothesis (H1) that the response pattern of the means from the SO_2 exposed group was the same as the response pattern of the means from the air exposed group. Since hypothesis H1 is to be tested on 17 parameters measured in this one experiment, the Bonferroni inequality was used to calculate the p-value at or below which H1 can be rejected. This procedure ensures an experiment-wise error rate of 0.05 or less (the level of significance chosen for hypothesis testing in this experiment). The p-value obtained by this method is 0.003 ($0.05 \div 17 = 0.003$). Actually, the correct p-value to use for testing H1 for each parameter lies somewhere between 0.05 and 0.003. The value 0.003 will apply only if the tests of H1 for the 17 parameters were independent since the Bonferroni correction is exact in this case. On the other hand, the value of 0.05 will be acceptable only if the tests of H1 for the 17 parameters were dependent to the point of being redundant. In our case, there is dependence of varying degrees so the correct p-value lies somewhere between the two extremes. We will therefore refer to tests with $p < 0.003$ as statistically significant ($p < 0.05$) and to tests with $0.05 \leq p \leq 0.003$ as possibly statistically significant.

SECTION 3

RESULTS

HEMATOLOGIC ANALYSIS

Table 1 gives the P values of the eleven individual tests for hypothesis (H1). Detailed values for each test are found in Tables 2 and 3. There was no significant difference between the air and the SO_2

TABLE 1. STATISTICAL P VALUES OF HEMATOLOGIC TESTS FOR HYPOTHESIS (H1) FROM SUBJECTS EXPOSED TO CLEAN AIR OR TO 0.75 PPM SO_2

Blood Parameter	H1 P
Red blood cells (RBC's)	.556
Mean cell volume (MCV)	.253
Hematocrit (Hct)	.980
Hemoglobin (Hgb)	.961
White blood cells (WBC's)	.674
Segmented neutrophils	.824
Bands	.774
Lymphocytes	.510
Monocytes	.005
Eosinophils	.722
Basophils	.964

TABLE 2. MEAN HEMATOLOGIC VALUES FROM SUBJECTS EXPOSED TO CLEAN AIR (N = 14)

Parameter	Time of Measurement				
	Pre-exposure (S.E.)	Immediately post-exposure (S.E.)	24 hr Post-exposure (S.E.)	48 hr Post-exposure (S.E.)	72 hr Post-exposure (S.E.)
RBC's ($x10^6/mm^3$)	5.20 (.08)	5.20 (.07)	5.19 (.07)	5.16 (.04)	5.12 (.05)
MCV (μ^3)	87.9 (.8)	86.5 (.6)	88.0 (.7)	87.4 (.7)	88.2 (.6)
Hct (%)	45.0 (.6)	44.2 (.6)	45.0 (.6)	44.4 (.4)	44.4 (.4)
Hgb (g/100 ml)	15.7 (.2)	15.8 (.2)	15.6 (.2)	15.6 (.1)	15.4 (.2)
WBC's ($x10^3/mm^3$)	5887 (252)	5716 (241)	5930 (224)	5600 (347)	5719 (281)
Neutrophils, segmented (%)	52.6 (2.3)	56.4 (1.9)	54.1 (1.9)	51.5 (1.4)	51.3 (1.7)
Neutrophils, banded (%)	.71 (.22)	.50 (.36)	.50 (.23)	1.00 (.43)	.79 (.35)
Lymphocytes (%)	37.8 (2.0)	36.4 (1.7)	37.2 (2.2)	37.4 (1.3)	38.2 (1.6)
Monocytes (%)	5.2 (.7)	4.5 (.7)	4.7 (.6)	6.4 (.4)	5.7 (.5)
Eosinophils (%)	3.4 (.8)	2.0 (.6)	3.1 (.7)	3.5 (.6)	3.8 (1.1)
Basophils, (%)	.29 (.16)	.14 (.14)	.14 (.10)	.21 (.11)	.21 (.15)

TABLE 3. MEAN HEMATOLOGIC VALUES FROM SUBJECTS EXPOSED TO 0.75 ppm SO_2 (N = 14)

Parameter	Time of Measurement				
	Pre-exposure (S.E.)	Immediately post-exposure (S.E.)	24 hr Post-exposure (S.E.)	48 hr Post-exposure (S.E.)	72 hr Post-exposure (S.E.)
RBC's ($x10^6/mm^3$)	4.96 (0.09)	4.95 (.07)	4.91 (.09)	4.86 (.09)	4.77 (.08)
MCV (μ^3)	88.7 (.6)	87.6 (.7)	89.4 (.6)	89.4 (.6)	90.6 (1.0)
Hct (%)	42.9 (.7)	42.5 (.4)	43.0 (.7)	42.6 (.8)	42.7 (.5)
Hgb (g/100 ml)	15.1 (.2)	15.1 (.2)	15.0 (.2)	15.0 (.2)	14.8 (.2)
WBC's ($x10^3/mm^3$)	6025 (382)	5899 (417)	5744 (302)	5481 (367)	5609 (229)
Neutrophils, segmented (%)	56.9 (1.3)	57.5 (1.4)	56.3 (1.4)	54.4 (1.7)	54.3 (1.5)
Neutrophils, banded (%)	0.57 (.17)	0.50 (.23)	0.50 (.14)	0.43 (.20)	0.43 (.20)
Lymphocytes (%)	34.2 (1.4)	34.4 (1.4)	34.5 (1.3)	37.1 (1.5)	36.1 (1.4)
Monocytes (%)	5.8 (.4)	5.9 (.4)	6.4 (.4)	4.8 (.5)	6.6 (.5)
Eosinophils (%)	2.4 (.5)	1.7 (.4)	2.3 (.5)	3.1 (.6)	2.6 (.4)
Basophils, (%)	0.14 (.10)	0.07 (.07)	0.07 (.07)	0.21 (.11)	0.07 (.07)

TABLE 4. MEAN PROLIFERATIVE RESPONSES OF LYMPHOCYTES TO PHA OR PWM FROM HUMANS EXPOSED TO CLEAN AIR OR TO 0.75 ppm SO_2

	Air (N = 6)						SO_2 (N = 4)					
Time of measurement	No mitogen		PHA (2 µg/ml)		PWM (2.5 µl/ml)		No mitogen		PHA (2 µg/ml)		PWM (2.5 µl/ml)	
	cpm	S.E.	cpm	S.E.	cpm	S.E.	cpm	S.E.	cpm	S.E.	cpm	S.E.
Pre-exposure	373	160	29,673	5,889	8,637	2,064	139	34	19,872	5,836	11,464	3,956
24 hr post-exposure	366	66	33,383	1,856	13,861	3,314	103	30	19,488	5,687	12,329	1,753
48 hr post-exposure	385	93	32,925	1,889	12,741	2,940	213	119	24,334	1,454	7,684	638
72 hr post-exposure	338	135	34,578	2,061	12,158	4,282	430	237	19,968	3,354	13,701	3,030

P = 0.542 for no mitogen; P = 0.420 for PHA, P = 0.227 for PWM.

group means in the erythrocyte (RBC) counts, cell differential, or the associated parameters (MCV, Hct, Hgb) at any of the four post-exposure sampling times. A possibly significant decrease was found in the percentage of monocytes in the SO_2 group compared to controls at 48 hours post-exposure (Table 1; $p = 0.005$; $4.8 \pm 0.5\%$; $6.4 \pm 0.4\%$). This change paralleled a progressively decreasing mean total leukocyte (WBC) count which was decreased to $5481 \pm 367/mm^3$ at 48 hours from $6025 \pm 382/mm^3$ at 0 hour. Both the leukocyte and the monocyte values had slightly recovered in samples taken at 72 hours post-SO_2 exposure. It may be noted that at 48 hours the percentage of lymphocytes was increased in the SO_2 group ($34.2 \pm 1.4\%$ at 0 hour; $37.1 \pm 1.5\%$ at 48 hours).

Lymphocyte Transformation

Lymphocyte transformation included data from only 6 subjects in the air group and 4 subjects in the SO_2 group. Table 4 shows no significant differences between the SO_2 and the air group means in the responsiveness of lymphocytes to PHA or PWM. The baseline data of unstimulated lymphocyte cultures (without mitogens) showed no significant differences.

Lymphocyte Rosettes

The results (Table 5) of active T-lymphocyte numbers bearing membrane receptors for sheep erythrocytes (TEa rosettes) showed no significant differences between the means of the SO_2 and the air groups ($p = 0.746$), however, the TEa values in the SO_2 group continued to decrease after the exposure including the 72 hour sample (567 ± 75 at 0 hour; 417 ± 63 at 72 hours). In contrast, the mean TEa cell number for the air group showed a partial recovery at 72 hours (656 ± 81 at 0 hour; $581 \pm$ at 72 hours).

No significant differences were found between the air and SO_2 group means in the lymphocytes bearing receptors for the Fc portion of IgG (Table 6; $p = 0.227$). Examination of this table, however, indicates an increase of Fc cells at 48 and 72 hours in the SO_2 group (363 ± 42 at 0 hour; 411 ± 45 at 48 hours; 420 ± 45 at 72 hours) in contrast to a decrease in the air groups 423 ± 54 at 0 hour; 360 ± 55 at 48 hours; 359 ± 42 at 72 hours).

SECRETORY IgA

No significant differences were observed in the s-IgA means between the air and SO_2 groups (Table 7; $p = 0.279$). The s-IgA levels in the SO_2 group, however, remained elevated throughout the post-exposure sampling period (0.455 ± 0.074 at 0 hours; 0.776 ± 0.220 at 48 hours).

TABLE 5. MEAN ACTIVE ROSETTING LYMPHOCYTE NUMBERS IN SUBJECTS EXPOSED TO CLEAN AIR OR TO 0.75 ppm SO_2

Time of measurement	Air (N = 11) Mean	S.E.	SO_2 (N = 13) Mean	S.E.
Pre-exposure	656	81	567	75
24 hrs post-exposure	582	154	518	66
48 hrs post-exposure	500	114	441	40
72 hrs post-exposure	581	76	417	63

P = 0.746.

TABLE 6. MEAN FC ROSETTING LYMPHOCYTE NUMBERS IN HUMANS EXPOSED TO CLEAN AIR OR TO 0.75 ppm SO_2

Time of measurement	Air (N = 11) Mean	S.E.	SO_2 (N = 13) Mean	S.E.
Pre-exposure	423	54	363	42
24 hrs post-exposure	423	50	346	41
48 hrs post-exposure	360	55	411	45
72 hrs post-exposure	359	42	420	45

P = 0.227.

TABLE 7. MEAN SECRETORY IgA VALUES (mg/dl) FROM HUMANS EXPOSED TO CLEAN AIR OR TO 0.75 ppm SO_2

Time of measurement	Air (N = 7) Mean	S.E.	SO_2 (N = 11) Mean	S.E.
Pre-exposure	.647	.106	.455	.074
24 hrs post-exposure	.721	.155	.673	.181
48 hrs post-exposure	.697	.181	.776	.220
72 hrs post-exposure	.413	.089	.619	.178

P = 0.279.

SECTION 3

DISCUSSION

The hematologic data show that one 2 hour exposure to 0.75 ppm SO_2 (with a brief treadmill exercise) has no effect on the number of peripheral blood erythrocytes or the associated parameters (Hct, MCV, Hgb) in healthy male subjects.

A possibly significant decrease was found in the mean percentage of monocytes 48 hours after the SO_2 exposure ($p = 0.005$). It is possible that in the time period involved, there was a sequestration of leukocytes from capillaries into the irritated site, the lung, and the airway epithelium as a normal response of the host defense system (Senn and Jungi, 1975). Monocytes are known to migrate from blood into tissues to become phagocytes in response to an inflammatory stimulus (von Furth and Cohn, 1968). This change is not considered to be biologically significant because it is not greater than normal daily variation among individuals.

The analysis of the immunologic data showed no statistically significant differences between the means of the SO_2 and the air groups for any of the immunologic parameters tested up to and including the 72 hour post-exposure sampling. However, this study showed a decrease in TEa and an increase in Fc receptor bearing lymphocytes, which persisted at 72 hour post SO_2 exposure period. The active T cells appear to be a sub-population of lymphocytes that is actively involved in cell mediated immunity and a decrease in their values may represent a functional T cell defect (Wybran, et al., 1973). The sub-population of lymphocytes with Fc-IgG receptors has been demonstrated to include suppressor cells, which mediate cytotoxic reactions (Moretta, et al., 1977) and control the functions of B and other T cells (Gupta, 1978). Hyperactivity of suppressor cells has been seen in individuals with immunodeficiency (Gupta and Good, 1977). The importance of the changes in the two T subsets noted at 72 hours post SO_2 exposure should be assessed through repeated studies. It would be of interest to expand the follow-up sampling period.

The study presented provides some suggestive evidence that exposure to SO_2 may transiently affect the circulatory blood cells. The data, however, give no information on individual responses nor on the mechanism by which the effect is brought about. This study was performed using young, healthy, male subjects and consequently provides no information on the possible effects of SO_2 on other segments of the population which might be more susceptible to this air pollutant.

The study suggests that future research should include immunologic assays to measure pollutant-induced changes in lymphoid cell numbers and functions which are considered as early indicators of cellular injury. Measurements should be made in populations at risk such as asthmatics using SO_2 combinations with other pollutants to resemble atmospheric conditions. Finally, the follow-up sampling should be extended beyond 72 hours post-exposure period to allow an immunologic response to develop.

REFERENCES

Anderson, I., G. R. Lundquist, P. L. Jensen, and D. F. Proctor. 1974. Human Response to Controlled Levels of Sulfur Dioxide. Arch. Environ. Health. 28:31-37.

Baier, R. E. 1975. In: Symposium in Applied Chemistry at Protein Interfaces, Advances in Chemistry, Series 145, Am. Chem. Soc., Washington, D.C.

Douglas, J. W. B. and R. E. Waller. 1966. Air Pollution and Respiratory Infection in Children. Br. J. Prev. Soc. Med. 20:1-8.

Ferris, B. G., Jr., I. T. T. Higgins, M. W. Higgins, J. M. Peters, W. F. Van Gamse, and M. D. Goldman. 1971. Chronic Non-specific Respiratory Disease. Berlin, New Hampshire, 1961-1967: A Cross-sectional Study. Amer. Rev. Resp. Dis. 104:232-244.

Ferris, B. G., Jr., I. T. T. Higgins, M. W. Higgins, and J. M. Peters. 1973. Sulfur oxides and suspended particulates: Possible Effects of Chronic Exposure. Arch. Environ. Health. 27:179-182.

Ferris, B. G., Jr., H. Chen, S. Puleo, and R. L. H. Murphy, Jr. 1976. Chronic Non-specific Respiratory Disease in Berlin, New Hampshire, 1967-1973. Amer. Rev. Resp. Dis. 113:475-485.

Frank, N. R., R. E. Yoder, J. D. Brain, and E. Yokoyama. 1969. SO_2(^{35}S Labeled) Absorption by the Nose and Mouth Under Conditions of Varying Concentration and Flow. Arch. Environ. Health. 18:315-322.

Geller, I., N. D. Greene, and C. J. Allen-Rowlands. 1978. Development of Bioindicators to NO_2 and SO_2 Exposures. EPA Contract 68-02-2279.

Gunnison, A. F. and A. W. Benton. 1971. Sulfur Dioxide: Sulfite. Interaction with Mammalian Serum and Plasma. Arch. Environ. Health. 22:381-388.

Gunnison, A. F. and E. D. Palms. 1973. Persistence of Plasma S-Sulfunates Following Exposure of Rabbits to Sulfite and Sulfur Dioxide. Toxic Appl. Pharmacol. 24:266-278.

Gupta, S. 1978. Functionally Distinct Subpopulations of Human T Lymphocytes - A Review. Clin. Bul. 8:100.

Gupta, S. and R. A. Good. 1977. Subpopulations of Human T Lymphocytes. I. Studies of Immuno-deficient Patients. Clin. Exp. Immunol. 30:222.

Hayatsu, H. and R. C. Miller, Jr. 1972. The Cleavage of DNA by the Oxygen-dependent Reaction of Bisulfite. Biochem. Biophys. Res. Commun. 46:120-124.

Henry, J. B. 1979. Todd-Sanford-Davidson Clinical Diagnosis and Management by Laboratory Methods. W. B. Saunders Company, Philadelphia. pp. 858-917.

Lawther, P. J., R. E. Waller, and M. Henderson. 1970. Air Pollution and Exacerbations of Bronchitis. Thorax. 25:525-539.

Lunn, J. E., J. Knowelden, and A. J. Handyside. 1967. Patterns of Respiratory Illness in Sheffield Infant Schoolchildren. Br. J. Prev. Soc. Med. 21:7-16.

Lunn, J. E., J. Knowelden, and R. W. Roe. 1970. Patterns of Respiratory Illness in Sheffield Junior Schoolchildren: A Follow-up Study. Br. J. Prev. Soc. Med. 24:223-228.

Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard and M. D. Cooper. 1977. Functional analysis of two human T-cell sub-populations: help and suppression of B-cell responses by T cells bearing receptor for IgM and IgG. J. Exp. Med. 146:184-200.

Oppenheim, J. J. and B. Schecter. 1976. In: Manual of Clinical Immunology, N. R. Rose and H. Friedman, eds. American Society for Microbiology, Washington, D.C.

Rall, D. P. 1974. Review of the Health Efforts of Sulfur Oxides. Environ. Health Perspec. 8:97-121.

Senn, H. J. and W. F. Jungi. 1975. Neutrophil Migration in Health and Disease. Semin. Hematol. 12:27.

Schneider, L. K. and G. A. Calkins. 1971. Sulfur Dioxide Induced Lymphocyte Defects in Human Peripheral Blood Cultures. Environ. Res. 3:473-484.

Shapiro, R. 1977. Genetic Effects of Bisulfite (Sulfur Dioxide). Mutat. Res. 39:149-175.

Speizer, F. E. and N. R. rank. 1966. The Uptake and Release of SO_2 by the Human Nose. Arch. Environ. Health. 12:725-728.

Strong, A. 1978. Description of the CLEANS Human Exposure Chamber. EPA-600/1-78-064, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina.

Van der Lende, R., C. Huygen, E. J. Jansen-Koster, S. Knijpstra, R. Peset, B. F. Visser, E. H. E. Wolfs, and N. G. M. Orie. 1975. A Temporary Decrease in Ventilatory Function of an Urban Population During an Acute Increase in Air Pollution. Bull. Physiopathol. Respir. 11:31-43.

van Furth, R. and Z. A. Cohn. 1968. The Origin and Kinetics of Mononuclear Phagocytes. J. Exp. Med. 128:415.

Wybran, J., A. S. Levin, L. E. Spitler, and H. H. Fudenberg. 1973. Rosette-forming Cells, Immunologic Deficiency Diseases and Transfer Factor. New England J. Med. 288:710-713.

Yokoyama, E., R. E. Yoder, and N. F. Frank. 1971. Distribution of ^{35}S in the Blood and Its Excretion in Urine of Dogs Exposed to $^{35}SO_2$. Arch. Environ. Health. 22:389-395.